EFFECTS OF THE SIZE AND FREQUENCY OF BLOOD MEALS ON *CIMEX LECTULARIUS* L.

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Successive small blood meals can induce moulting in C. lectularius. *Interval between meals is important in the effect on moulting. The food conversion efficiency in the different instars varies between 25.6 and 37%. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%. Unfed females did not lay eggs and the number of eggs laid per female as well as longevity showed good correlation with the amount of blood ingested.*

Accounts of the biology of *Cimex lectularius*, reared under various conditions, have been published by Bacot (1914), Hase (1917, 1919, 1930), Cragg (1923), Jones (1930), Kemper (1930), Janisch (1933, 1935), Kassianoff (1937), Johnson (1939, 1940a, 1940b, 1942), de Meillon and Golberg (1946, 1947), and Bell and Schaefer (1966). More recently Usinger (1966) published a monograph on Cimicidae reviewing most of the work done on this group of insects. Similarly studies on fertilization and physiology of reproduction of the adults have been published by Cragg (1915, 1920), Mellanby (1935, 1939a) and Davis (1964, 1965a, 1965b). Although a relationship between food supply and the growth of *C. lectularius* has been demonstrated (Titschack 1930 and de Meillon and Golberg 1946 and 1947), the effect of the size and frequency of blood meals has not been studied. This investigation was undertaken with a view to filling some gaps in the knowledge of bedbug biology.

METHODS

The threshold of hatching, nymphal development, and adult activity is between 13 and 15 C (Hase 1930, Mellanby 1935, Kemper 1936, Johnson 1942). The thermal death point is 44 C. Omori (1941) found that development ceased at 36 or 37 C. The temperature of 80 F (26.7 C) and 75% R.H. used for the stock culture proved to be in the range of optimum conditions for development, and was used for the experiments. The insects were kept in 4 x 4 x 1.5 cm plastic boxes with folded pieces of filter paper and were allowed to feed twice every week on human blood. The insects fed through the organdie covering a 3 cm diameter hole in the lid of the plastic box. The eggs were laid on the folded filter papers which were collected in new boxes where the eggs were allowed to hatch at the same temperature and relative humidity.

Two series of experiments were conducted for studying the effects of different blood meal sizes on *C. lectularius* .

In the first series newly moulted insects, taken from the standard culture, were kept singly in 2 x 7 cm specimen tubes with a 2 cm^2 piece of folded filter paper. The insects were fed only once on the second day after moulting. Feeding periods of five or six different lengths were used for each instar to provide the different meal sizes. For each feeding period 20 insects were used from every instar. Observations on subsequent moulting and longevity were recorded daily.

The efficiency of the various instars in converting human blood into body tissue and extra-cellular fluid was determined by the method given by Friend et al. (1965). The amount of blood required to produce 1 mg gain in body weight was calculated. The average difference in body weight between instars divided by the weight of the blood meal and multiplied by 100 is the food conversion efficiency % (Friend et al. 1965). Twenty first instar nymphs were taken from the standard culture immediately after hatching for determining the weight changes during development and the food conversion efficiency of the different instars. These insects were fed to capacity on the second day after hatching and once every ten days at least thereafter. The changes in body weight were recorded during development till the insects reached maturity. This way of estimating food conversion efficiency is subject to certain errors. Calculation of food conversion efficiency is always done on a dry weight basis, but is still subject to some errors. The most important of these arise from changes and differences in water content of both blood and insects, and from the presence of blood residues in the gut.

Newly moulted adults were used for studying the effects of different blood meal sizes on fecundity, longevity, and the duration of the preoviposition period. The following combinations of males and females and blood meal size were studied:

unfed female x engorged male
female fed for 60 seconds x engorged male
female fed for 120 seconds x engorged male
female fed for 240 seconds x engorged male
engorged female x unfed male
engorged female x engorged male
engorged female x male fed for 60 seconds
engorged female x male fed for 120 seconds
engorged female x male fed for 240 seconds.

For each combination 20 pairs were used and each pair was put in a single 2 x 7 cm specimen tube together with a 2 cm^2 piece of filter paper.

In the second series of experiments eggs were taken from the standard culture and put separately in 2 x 7 cm specimen tubes each with a piece of filter paper. The insects were fed on the second day after hatching. Eighty insects in four groups of 20 were used for the study of the effects of each feeding period. The four groups were given the blood meal of known duration at frequencies of 2, 4, 8, and 16 days respectively. Observations were carried out daily and the effects of the different feeding periods and their frequencies on the duration of the nymphal stadia, and on the preoviposition period; fecundity, longevity, and weight changes during development were recorded.

As most of the insects did not reach the adult stage when the feeding periods were 15, 60, or 120 seconds, at the different frequencies, an additional experiment was conducted. One hundred and sixty pairs in four groups of forty were taken from the standard culture as soon as they moulted from the fifth instar and used to complete the study of the effects of the size and frequency of blood meals on fecundity, longevity and the duration of the preoviposition period of the female and male. The feeding periods used were 15, 30, 60, and 120 seconds at the same

frequencies as before. Ten pairs were used for each frequency.

Effects of the Size of One Blood Meal

On the Nymphal Instars

The effects of the size of one blood meal per instar on per cent moulting, duration of the nymphal stadia, longevity and mortality rates were studied. Statistical analysis was undertaken to determine the level of correlation between the amount of blood ingested and each of duration of the nymphal stadia and longevity. A theoretical straight line relationship was assumed for the effect on the duration of the nymphal stadia and the longevity. The average decrement in the duration of the nymphal stadium and the average increment in the longevity per unit increase in the weight of the blood meal were estimated by calculating the coefficient of linear regression. The discrepancies between the observed values and the theoretical ones were shown by calculating the chi square to test the goodness of fit to the straight lines.

The effect of the size of one blood meal per instar on the percentage of nymphs which moulted in the different instars is shown in figs. 1a - e. No insect in any instar moulted if fed for 30 seconds or less. In all instars 100% moulting occurred if the insects were fed till engorgement. In all five nymphal instars there were positive correlations between the size of the blood meal and the percentage of nymphs which moulted; the correlation coefficients ranged between 0.916 and 0.999.

The minimum feeding period for a single blood meal to induce moulting was 60 seconds in the first and second instars, 40 seconds in the third instar, and 120 seconds in both the fourth and fifth instars. These periods are all less than half of the maximum feeding periods. Friend et al. (1965) stated that *R. prolixus* nymphs of all instars will moult normally when fed less than 50% of the maximum meal and for the third instar only 24.7% of the maximum was required. Locke (1958) caused fourth-instar nymphs of *R. prolixus* to moult by feeding them 35% of the maximum meal. The blood meal supplies the nutrients and stretches the abdomen. The latter initiates the hormone cycle that results in moulting (Friend et al. 1965). Wigglesworth (1963) demonstrated that nutrients alone do not stimulate moulting and this was supported by the work of Beckel and Friend (1964). The latter workers explained that release of the moulting hormone, and activation and division of the epidermis is caused by stretching and they claimed that in their experiments, because of either inadequate nutrient or some undiscovered factor, the moulting cycle was halted and the animal died prematurely. Locke (1958) studied the effect of the blood intake on the diameter of the tracheae produced. He found that the increase in the diameter at moulting in many of the tracheae in *Rhodnius* is proportional to the size of blood meal. From these studies on *C. lectularius* it was found that, in all the nymphal instars, moulting can be induced by blood meals much smaller than the full blood meal and that there is always a positive correlation between the size of the blood meal and the percentage of moulting insects.

Figs. 1a - e also show the effect of the size of the blood meal on the longevity of the different instars. The longevity of all five instars increased gradually with the increase in the size of the blood meal. The correlation coefficients ranged from 0.773 to 0.993. In the first four

instars chi square tests showed departure from a straight line relationship. In the fifth instar, on the other hand, the relationship proved to be a straight line.

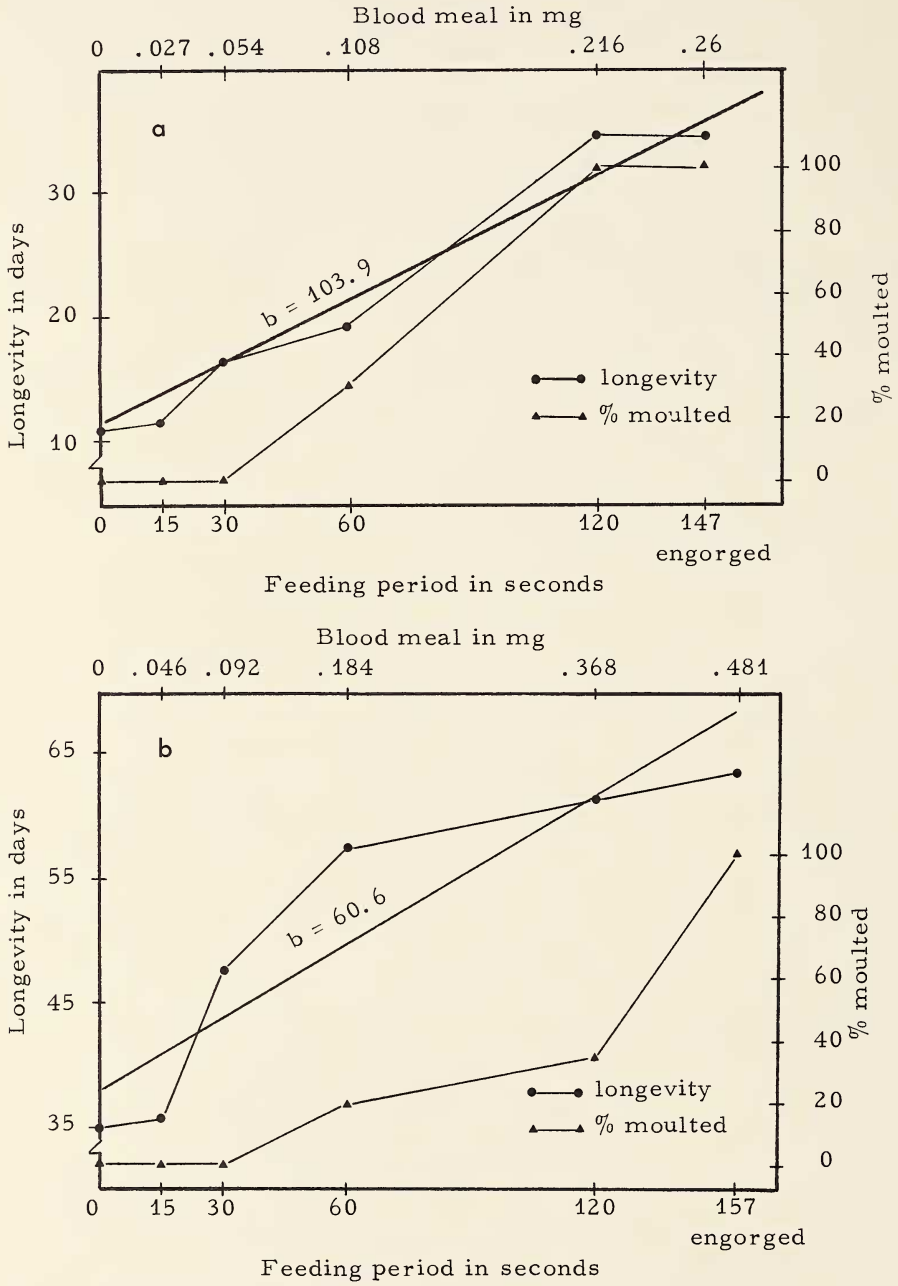
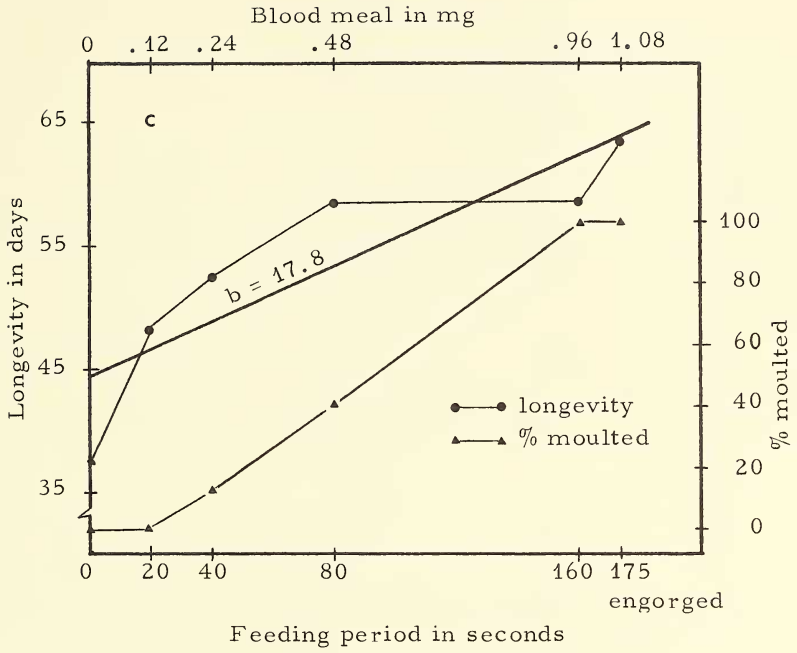


Fig. 1. Effect of the size of a single blood meal on the longevity and moulting of nymphs of *C. lectularius*. a. first instar; b. second instar.



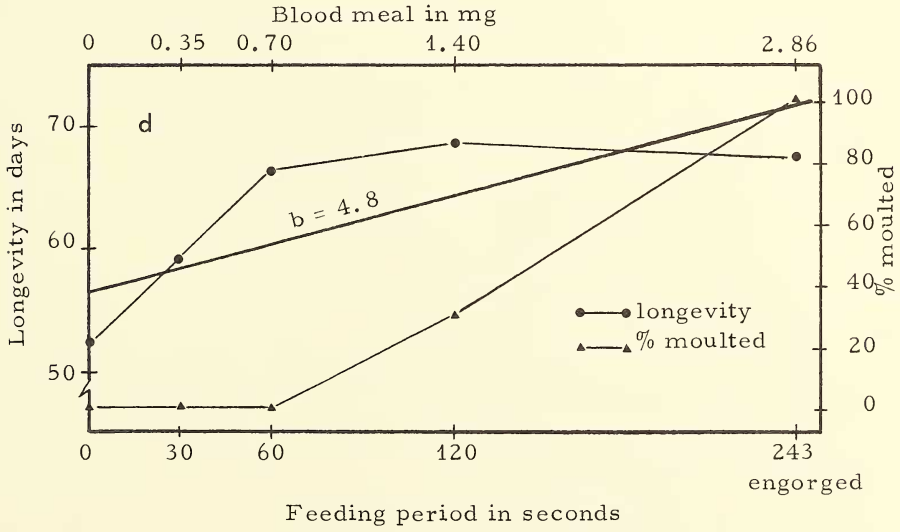


Fig. 1. c. third instar; d. fourth instar.

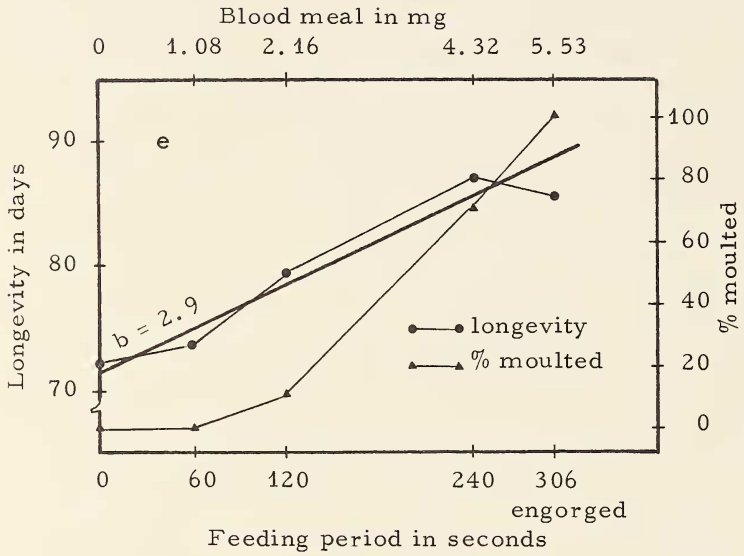


Fig. 1. e. fifth instar.

The effect of the size of the blood meal on the duration of the nymphal stadia was determined from those insects which moulted in each group. The duration of the nymphal stadium was taken as the period elapsing between the moult before feeding and that after feeding. The results are shown in table 1. In all the five nymphal instars there was a slight decrease in the duration of the nymphal stadium with the increase in the size of the blood meal. The correlation coefficient ranged between -0.79 and -0.99.

To estimate the effect of the size of a single blood meal on the mortality rate of the nymphal instars, the data were analyzed by probit method (Finney 1947). The LT50 was found for each blood meal size from the provisional probit line (figs. 2a to 2e). The reliability of the estimate is such that if the experiment is repeated there is 5% chance of getting an LT50 value that is not within the fiducial limits.

The LT50 values and their fiducial limits are shown in table 2. In all the five nymphal instars it was found that the LT50 increases with the increase in the size of the blood meal.

TABLE 1. Effect of the size of a single blood meal on the duration of the different stadia of *C. lectularius*.

Stadia:	Feeding period in seconds										
	0	15	20	30	40	60	80	120	160	240	engorged
1st											
x*	0	0.021		0.041		0.083		0.165			0.260
y**						5		4.7±0.15			4.5±0.13
	-	-		-		(6)		(20)			(20)
						5		4 - 5			4 - 5
2nd											
x	0	0.046		0.092		0.184		0.368			0.481
y						7		7			6.3±0.01
	-	-		-		(7)		(7)			(20)
						7		7			6 - 7
3rd											
x	0		0.124		0.248		0.496	0.992			0.087
y					7		7	6.4±0.01			5.6±0.01
	-		-		(2)		(8)	(20)			(20)
					7		7	6 - 7			5 - 6
4th											
x	0			0.035		0.704		1.408			2.856
y								7			6.6±0.01
	-			-		-		(6)			(20)
								7			6 - 7
5th											
x	0					1.086		2.172	4.345		5.533
y								8	6.2±0.08		6
	-					-		(2)	(14)		(20)
								8	6 - 7		6

* x = average weight of the blood meal in mg.

** y = mean duration of the stadium ± S.E.
(number of insects)
range

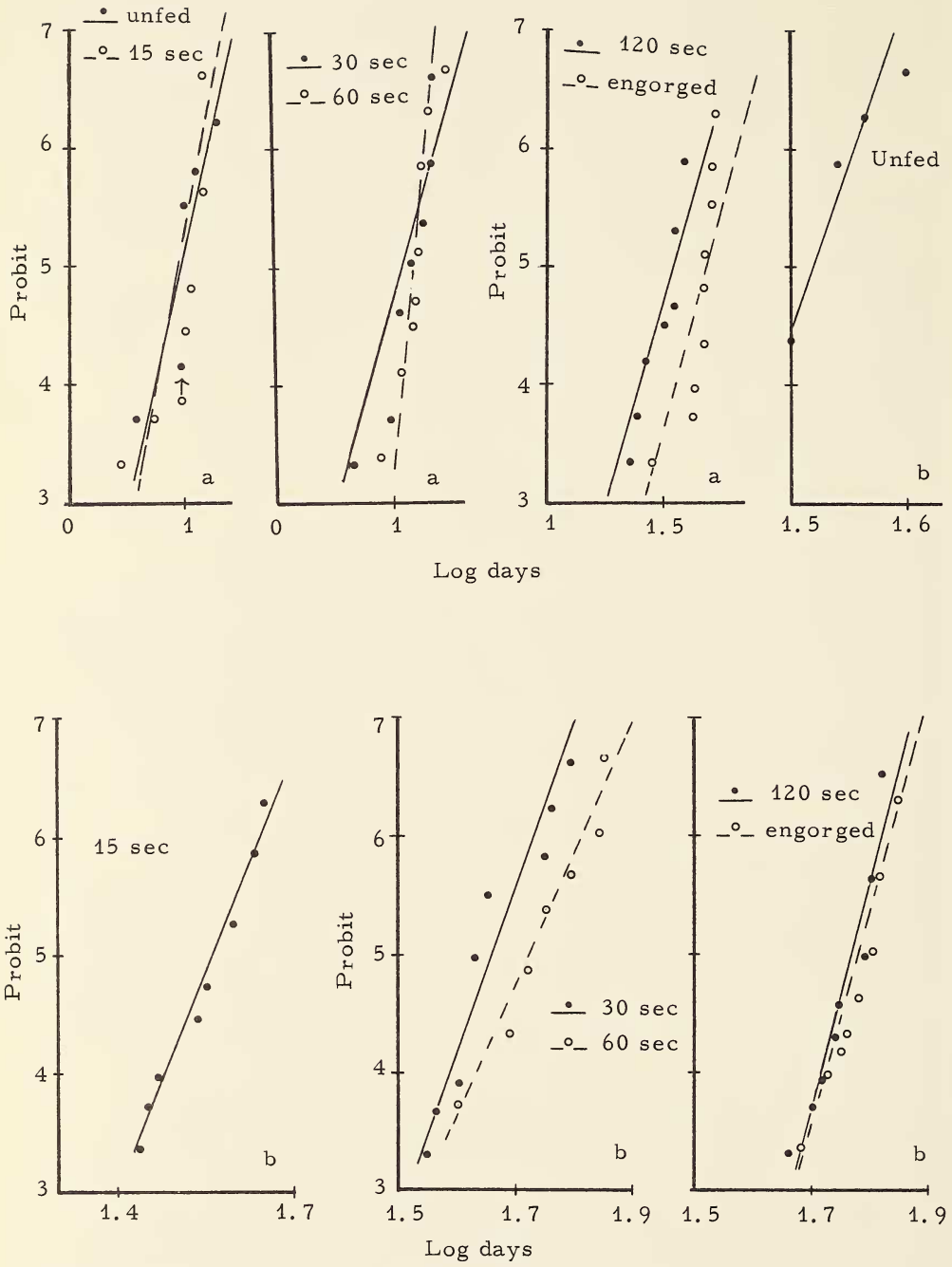


Fig. 2. Probit lines for longevity at various meal sizes in *C. lectularius*. a. 1st instar; b. 2nd instar.

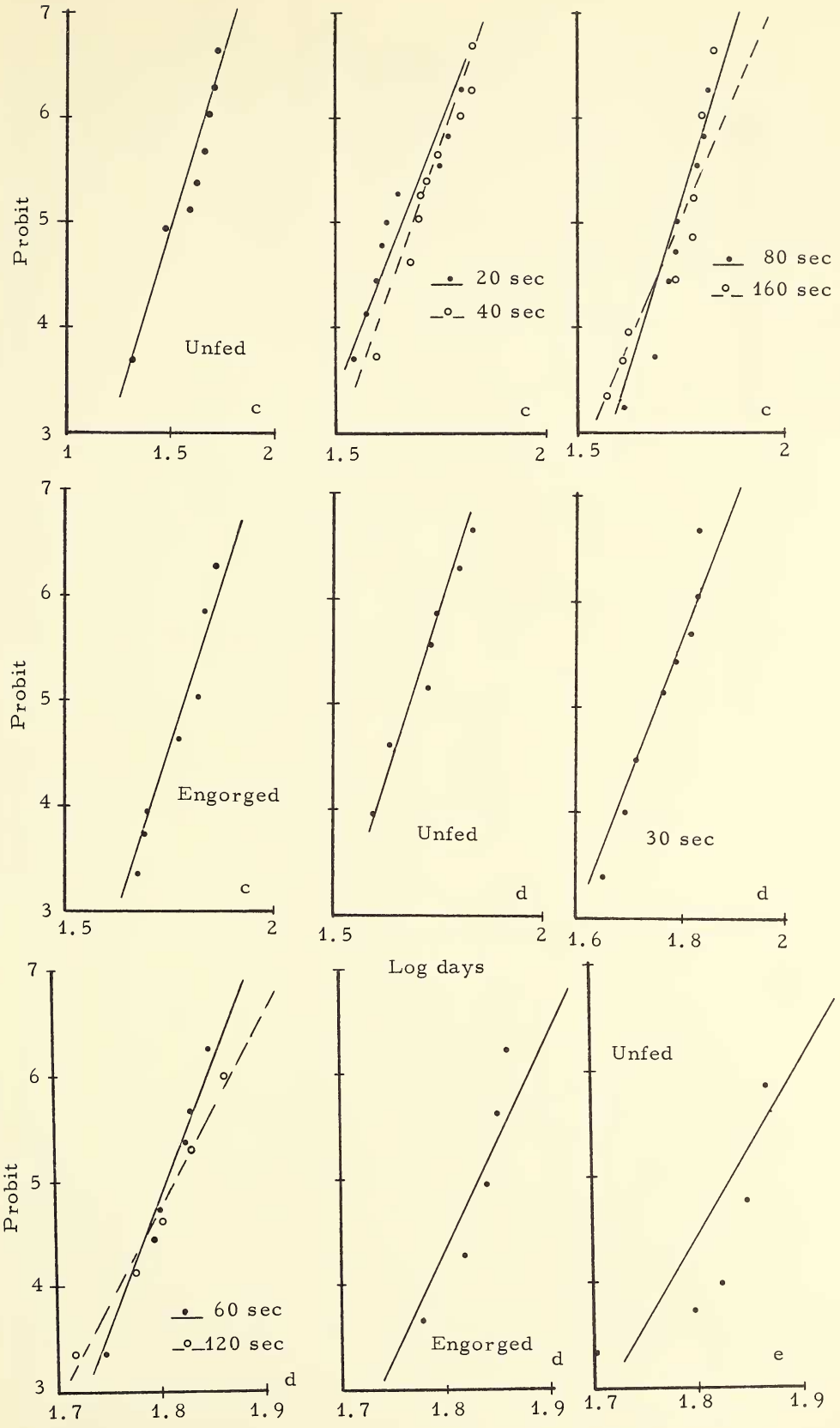


Fig. 2. c. 3rd instar; d. 4th instar; e. 5th instar.

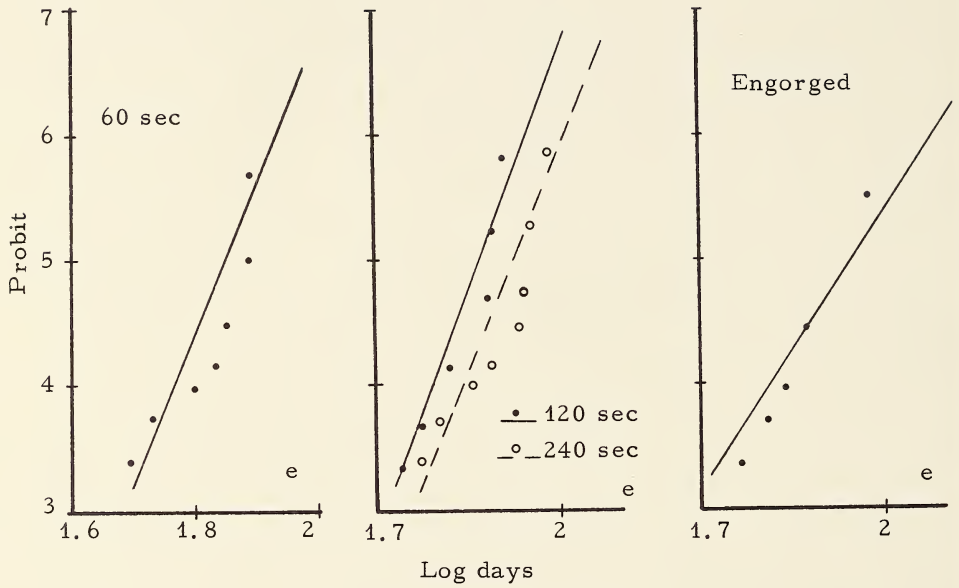


Fig. 2. e. 5th instar.

Fig. 3 shows the average weight changes during development of the nymphal instars when they take a full blood meal in each instar. The sudden rise in weight after taking the blood meal is followed by a rapid fall which is mostly due to the passage of the water and some other constituents of the blood during the first day after the meal. Over the period preceding the next meal the weight keeps dropping at a progressively slower rate. Titschack (1930) published a similar curve with small differences in weights. Przibram has proposed that the weight of an insect is doubled at each instar and the linear dimensions increased by 1.26 at each moult (Wigglesworth 1965). Although this rule is more applicable to Hemimetabola than to Holometabola, it is of doubtful value for *C. lectularius* because its growth curve appears discontinuous due to the ingestion of blood meals 3 to 5 times the body weight.

TABLE 2. Effect of the size of a single blood meal on the LT50 in the different instars of *C. lectularius*.

	Feeding period in seconds										
Instars	0	15	20	30	40	60	80	120	160	240	engorged
1st											
x*	0	1.021		0.042		0.083		0.165			0.260
y**	9.0	9.3		12.6		15.5		36.3			47.9
	(7.5-10.8)	(8.3-10.5)		(10.7-14.7)		(14.6-16.4)		(33.8-39.0)			(46.7-49.1)
2nd											
x	0	0.046		0.092		0.184		0.368			0.481
y	33.3	37.2		45.9		53.1		58.9			66.1
	(32.2-34.3)	(35.4-39.1)		(44.2-47.7)		(50.3-56.0)		(57.2-60.6)			(63.8-68.4)
3rd											
x	0		0.124		0.248		0.496		0.992		1.087
y	31.6		44.7		47.9		53.7		56.2		61.0
	(28.7-35.1)		(42.6-46.8)		(45.8-50.0)		(51.5-56.0)		(53.3-59.3)		(58.4-63.6)
4th											
x	0			0.352		0.704		1.408			2.856
y	48.4			58.9		64.4		65.3			27.6
	(46.2-50.8)			(57.1-60.7)		(63.0-66.0)		(63.1-67.7)			(65.9-69.4)
5th											
x	0					1.086		2.172		4.345	5.533
y	68.7					70.8		74.5		83.6	89.1
	(66.4-71.1)					(68.0-73.7)		(71.2-77.9)		(80.3-87.0)	(80.9-98.2)

* x = average weight of the blood meal in mg.
** y = LT50
(95% confidence limit)

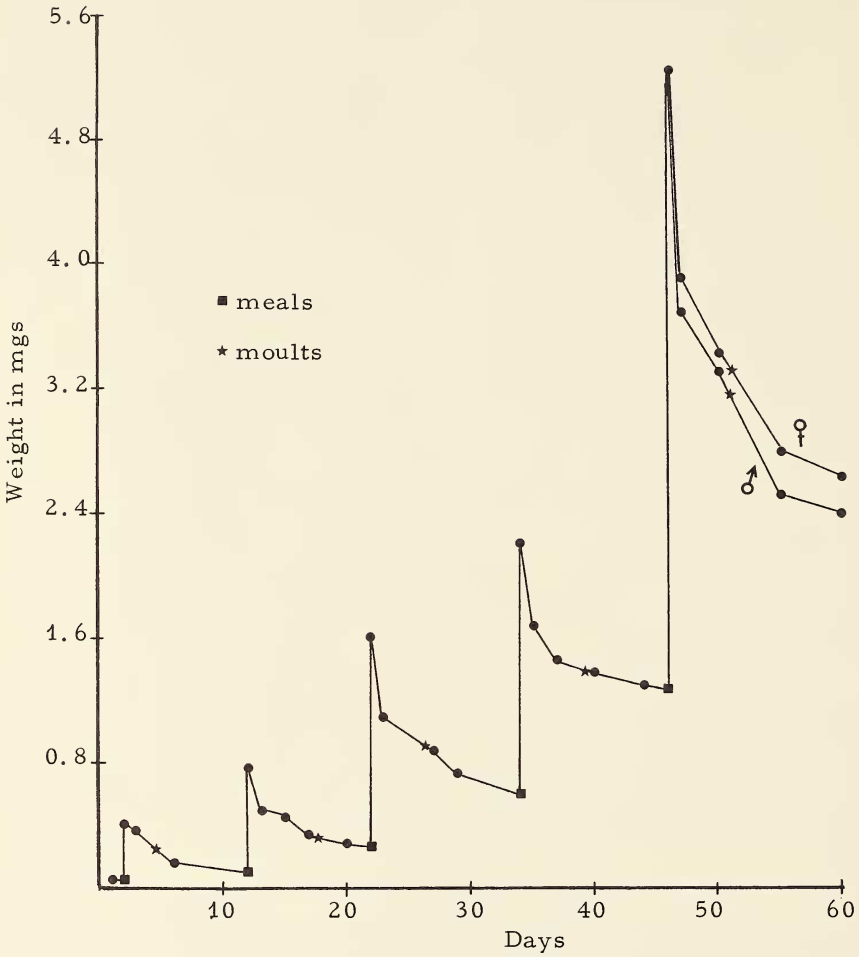


Fig. 3. Weight changes during development of *C. lectularius*.

Table 3 shows that the food conversion efficiency in the different nymphal instars of *C. lectularius* varies between 25.6 and 37.0%. It takes 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. The third instar is less efficient than are the others. Comparison of table 7 with the results of Jones (1930) shows a similarity in the trend of the food conversion efficiency in the different instars with small differences in the values. The values shown in Johnson's Table III (1960) are all higher than mine because he weighed the nymphs a short period after feeding. There is a difference in food conversion efficiency between the fifth instar and the adult male and female. This was overlooked by Jones (1930) and Johnson (1960).

TABLE 3. Food conversion efficiency in the nymphal instar of *C. lectularius*.

Moult*	Average difference in body wt. between instars (mg)	Average wt. of blood meal (mg)	Mg of human blood required to increase body wt. 1 mg	Food** conversion efficiency %
I to II	0.07	0.25	3.57	28.0
II to III	0.20	0.63	3.15	31.8
III to IV	0.27	1.02	3.90	25.6
IV to V	0.67	1.81	2.70	37.0
V to ♀	1.37	4.18	3.05	32.8
V to ♂	1.12	4.18	3.73	26.8
		Overall means:	3.70	30.3

* No. of insects was 20 in each test.

** Food conversion efficiency% = $\frac{\text{Average difference in body wt.}}{\text{Average wt. of blood meal}} \times 100$

On the Duration of the Preoviposition Period

The effect of the size of the blood meal on the duration of the preoviposition period is shown in figs. 4a and 4b. In fig. 4a the males were fed till engorgement and the females for different periods. No oviposition took place when the females were unfed. An increase in size of the female's blood meal caused a gradual decrease in the preoviposition period ($r = -0.908$). The highly significant value for chi square (49.8) shows departure from the theoretical straight line relationship.

In fig. 4b the females were fed till engorgement and the males for different periods. There was no significant difference in the duration of the preoviposition period.

On Fecundity

From fig. 5 it is clear that the size of the female's blood meal has a profound effect on the percentage of females which lay eggs when the mating males were fed till engorgement. No eggs were produced by unfed females. The more the females were fed the larger the number of females which laid eggs.

Fig. 6 shows no effect of the size of the mating males' blood meal on the percentage of females laying eggs when the latter were fed to capacity.

Figs. 5 and 6 also show the relationship between the size of the females' and mating males' blood meals and the number of eggs laid per female. Fig. 5 shows the effect of the size of the females' blood meal on the number of eggs laid per female when the mating males were fed to capacity. As shown in fig. 5 the more the females were fed the more eggs they laid.

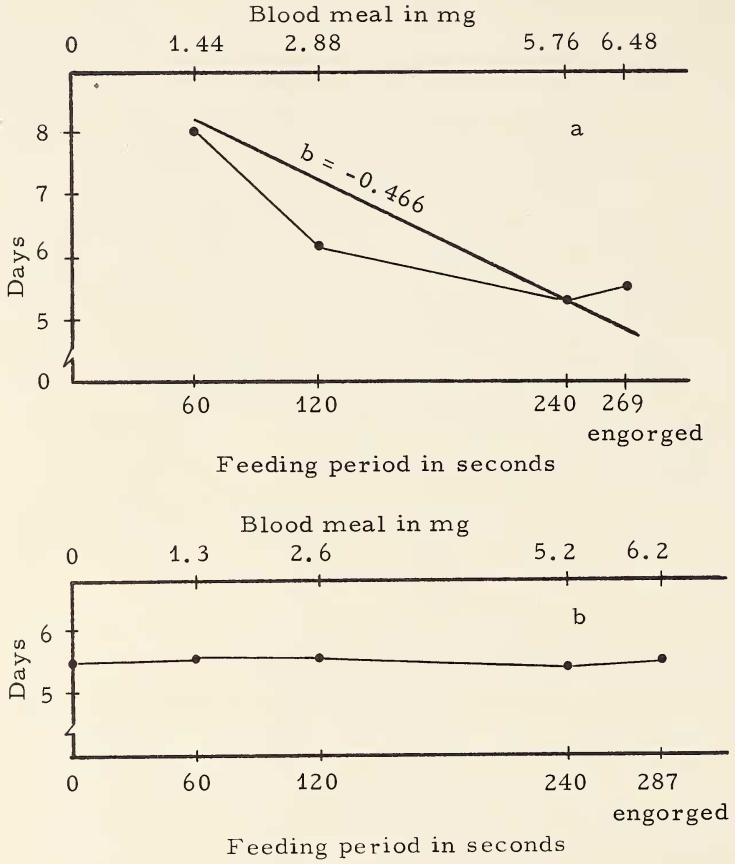


Fig. 4. a. Effect of the size of the female's blood meal on preoviposition period of *C. lectularius*; males engorged. b. Effect of the size of the mating male's blood meal on the preoviposition period of engorged female *C. lectularius*.

Fig. 5 also shows the relationship between the size of the females' blood meal and the number of eggs per milligram of blood. There is a slight increase in the number of eggs laid per milligram of blood with the increase in the size of the female's blood meal within the range of feeding periods from 60 and 240 seconds. On the other hand, there is a high increase when the feeding period increased from 240 to engorgement.

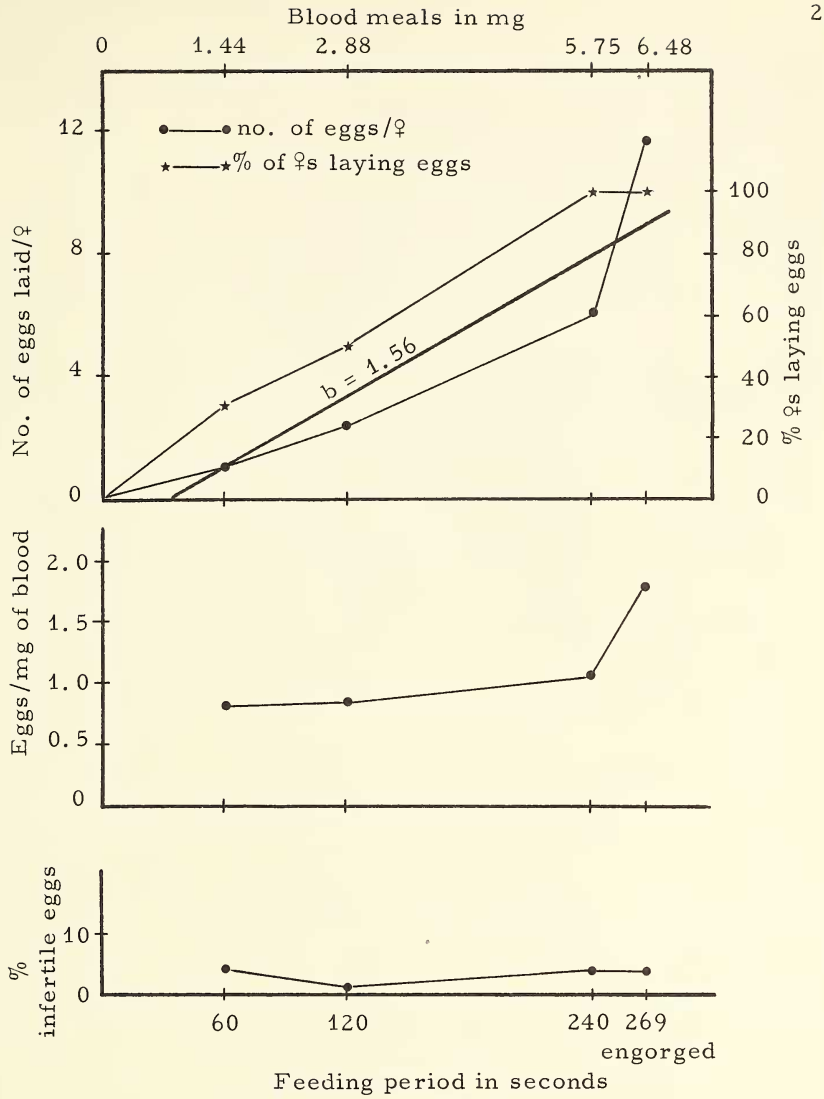


Fig. 5. Effect of the size of the blood meal of female *C. lectularius* on the percentage laying eggs, number of eggs laid per female, number of eggs laid per mg of blood, and percentage of infertile eggs.

Egg laying in *C. lectularius* has been studied under various conditions (Hase 1930, Titschack 1930, Omori 1941, Johnson 1942). In most blood-sucking insects, the number of eggs produced by a female is correlated, within limits, with the quantity of food taken (Wigglesworth 1960). The results shown in fig. 5 indicate that *C. lectularius* is no exception to this rule. Cragg (1923) wrote that the number of eggs is dependent on the amount of food obtained by the males as well as the females, and that females impregnated by unfed males do not produce as many eggs as females impregnated by fully nourished males. My studies confirm the

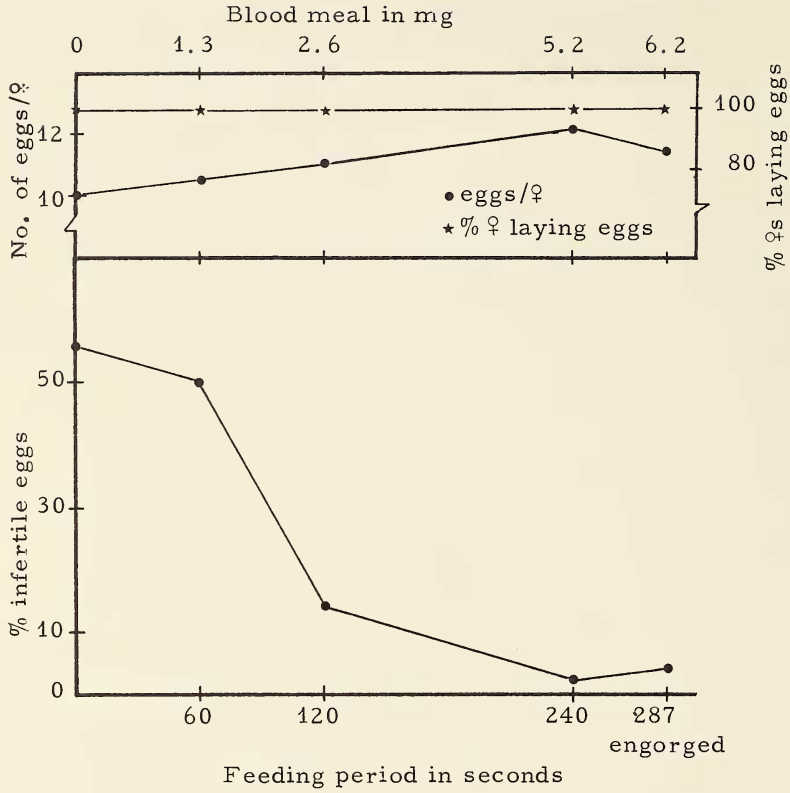


Fig. 6. Effect of the size of the mating males' blood meal on the percentage of females laying eggs, number of eggs, and the percentage of infertile eggs laid by engorged female *C. lectularius*.

former statement but indicate that the latter should be modified to read "fertile eggs". My unfed females did not lay eggs, although it was reported by Titschack (1930) and Johnson (1942) that unfed females can lay eggs. Jones (1930) claimed that the number of eggs produced by unfed females is probably related to the food reserves acquired in the previous instar. Davis (1964) studied the vitellogenesis of *C. lectularius* female that had mated but were starved and he found that no maturation occurs. Gooding (1963) postulated that although the most obvious function of the meal in human lice is that it provides the material and energy for growth of the nymphs and the maturation of eggs in the adult stage, it may also function as a stimulant resulting in the formation and release of hormones. Johansson (1964) said "after an adequate meal the 'nervous?' stimuli from the gut activate the corpus allatum by way of the brain.

Hormones from the corpus allatum and the neurosecretory cells in the brain stimulate the ovaries and accessory glands. In the absence of adequate food the brain inhibits the corpus allatum and the hormone titre remains too low for the ovaries and accessory glands to function normally." In *C. lectularius* it seems that there are more subtle factors involved in the production of eggs than mere quantities of blood taken. Bell and Schaefer (1966) found that the highest average egg production in *C. lectularius* (5.4) was by those fed on 9:1 mixture of whole rabbit blood and insect Ringer's, and the minimum was with females fed on Ringer's alone.

Figs. 5 and 6 show that although the size of the female's blood meal has no significant effect on the percentage of infertile eggs laid, the size of the blood meal of the mating male has a significant effect on the percentage of infertile eggs laid by the female.

Titschack (1930) found that the percentage of sterile eggs increased from 0 to 1% during the fertile period of *C. lectularius* . Mellanby (1939a) found that mating is a necessary process for egg production. Davis (1965b) studied the effect of insemination in activating the corpus allatum of female *C. lectularius* and he found that the mechanical aspects of insemination play no role in this activation and concluded that the presence of spermatozoa in the conceptacula or lateral oviducts stimulates the corpus allatum. He found that 3 to 4 hours are required for seminal stimulus to become effective and this period corresponds with the time required for the spermatozoa to reach the genital tract. He also suggested that the seminal stimulus results from summation of stimuli of many receptors in the genital tract.

On Longevity of the Adult

The effect of the size of the blood meal on the longevity of the adult female is shown in fig. 7. Analysis of the data was undertaken on the basis of dividing. The results were divided into females which did not lay eggs, females which laid eggs, and both together; the correlation coefficients were 0.99, 0.91 and 0.97 respectively. The straight line relationship for females which did not lay eggs and for all females did not hold for females which laid eggs. This indicates that when the females take smaller blood meals, egg production may have an influence on their longevity.

Fig. 7 also shows that the males lived longer when the blood meal was increased in size and that the relationship was a straight line.

Effects of the Size and Frequency of Blood Meals

On the Nymphal Instars

The effects of the size and frequency of the blood meals on moulting, duration of the nymphal stadia, and mortality were studied.

The size and frequency of blood meals have a profound effect on moulting of the insects in all instars (fig. 8). When the feeding period was 15 seconds on every 4th, 8th, or 16th day the first instar nymphs did not moult. At a frequency of every 2nd day only about 30% of the insects reached the second instar.

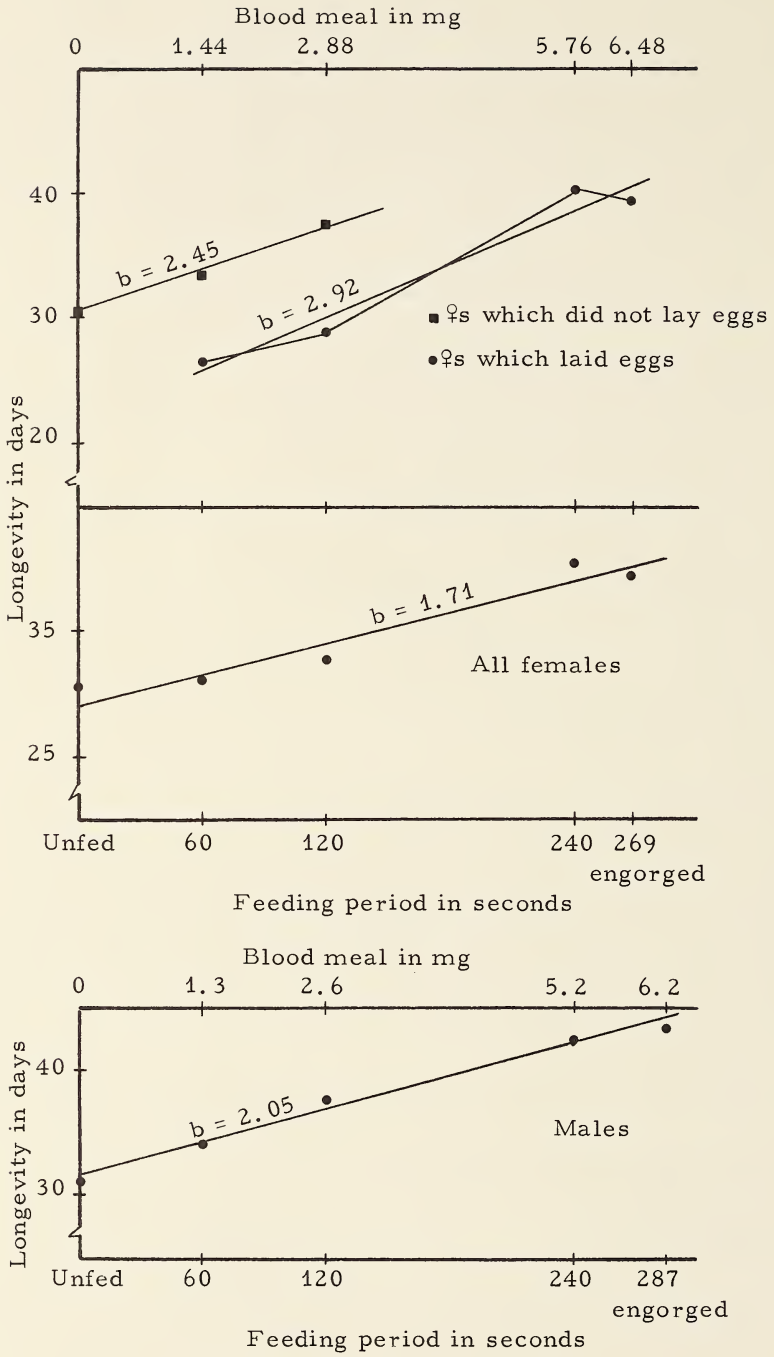


Fig. 7. Effect of the size of a single blood meal on the longevity of the female and male *C. lectularius*.

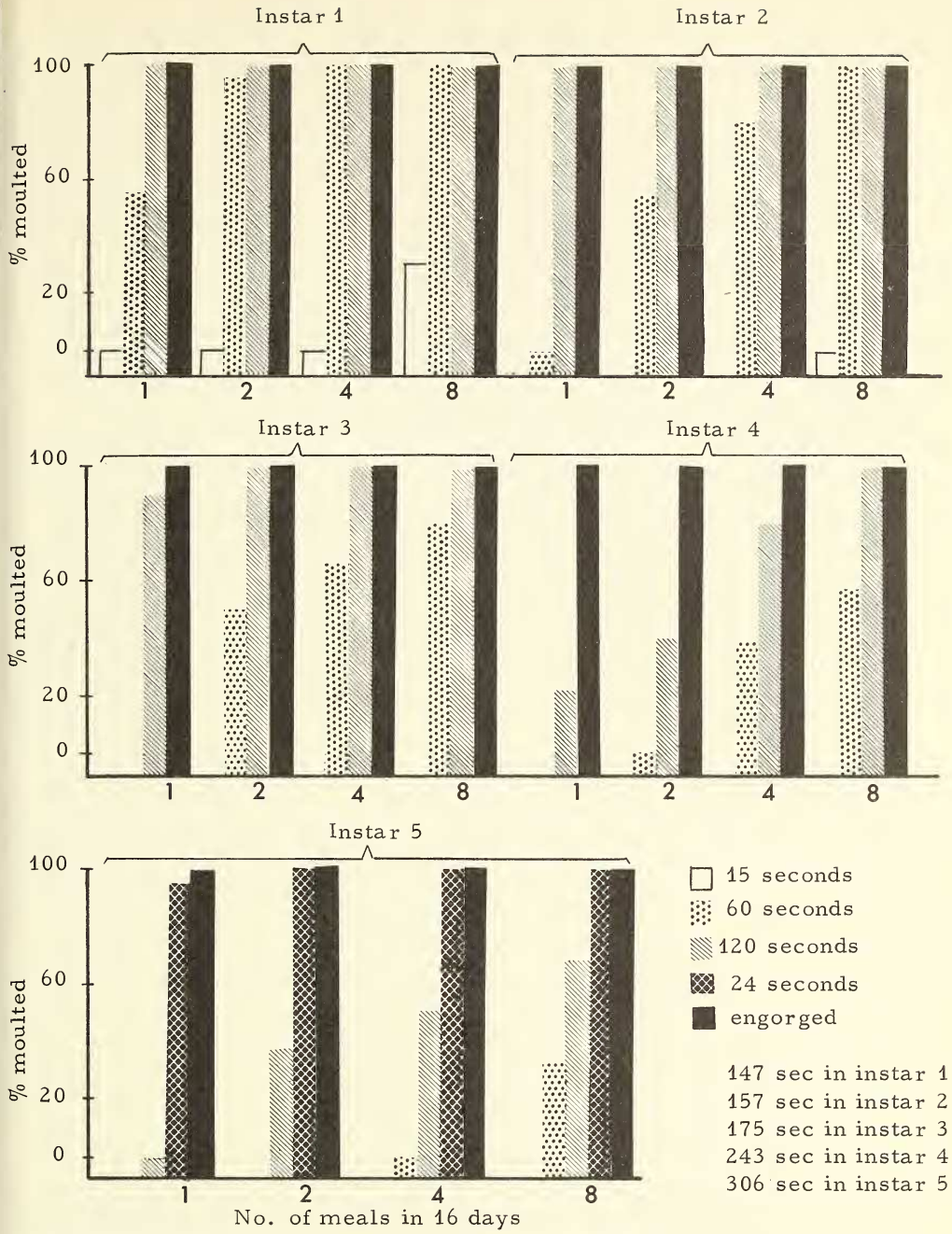


Fig. 8. Effect of the size and frequency of blood meals on moulting in the different instars of *C. lectularius*.

All insects fed for 60 seconds every 2 days reached the third instar but thereafter the number of nymphs that moulted decreased and only 15% of the insects reached the adult stage. When the interval between the blood meals was prolonged to 4 days all the insects reached the second instar and only 25% reached the fifth instar. At a frequency of every 8 days the number of insects that did not moult increased from the first to the fourth instar and 2.5% reached the fourth instar. When the insects were fed for 60 seconds every 16 days 55% reached the second instar.

All insects reached the third instar when fed for 120 seconds over the range of intervals between meals of 2 to 16 days. At a frequency of every 2nd day all the insects reached the adult stage. The number of insects that reached the adult stage decreased with the increase of the period between meals.

As the insects in the first four nymphal instar can engorge in less than 240 seconds, the effect of this feeding period and its frequency is shown in the fifth nymphal instar. For this experiment the insects were taken from the group which were fed till engorgement to the fourth instar at the different frequencies. When the fifth instar nymphs were fed for 240 seconds, in the range of frequencies studied, only one did not moult to the adult stage.

All insects which were fed till engorgement at any frequency reached the adult stage.

In general the number of insects which moult after the first meal at any instar increases with the increase in the size of the blood meal. Maximum moulting took place after taking the second meal, if the first meal did not bring about more than 50% moulting.

When the nymphs were given a series of blood meals, the percentage of moulting insects depended upon the size of blood meals as well as the intervals between them. Wigglesworth (1934) was unable to get the fifth instar nymphs of *R. prolixus* to moult by feeding them a series of small blood meals at intervals. Friend et al. (1965), on the other hand, found that third and fifth instar nymphs of *R. prolixus* can be induced to moult on a succession of small meals at 30 day intervals. Unfortunately they did not study the effect of the interval between feeding on moulting. My studies also show that successive small meals can induce moulting and that, in *C. lectularius*, the interval between feeding is very important. It seems that when the nymphs are fed a series of small meals within certain interval limits they achieve a physiological stage after which moulting can be induced by blood meals of small size. Friend et al. (1965) claimed that this might be due to some moulting hormone produced under the influence of small blood meals and stored until it reaches an effective titre when moulting results. They added that the cells may become more responsive to the moulting hormone after they have assimilated the nutrients supplied by small meals. This might be so in *C. lectularius* because the size and frequency of blood meals has no significant effect on the period between the last meal and the day of moulting.

The frequency of feeding has a profound effect on moulting and this effect is dependent on the size of the blood meal (fig. 8). The smaller the blood meal and the longer the interval between feeding the lower the percentage of the nymphs moulting. This may be due to the effect of the duration of the interval between feeding on the stored moulting hormone suggested by Friend et al. (1965).

The duration of any nymphal stadium increases with the increase in the number of meals required to induce moulting, and with the decrease of frequency of feeding. On the other hand, neither the size and interval between blood meals nor the number of meals have a significant effect on the period between the last meal and moulting. Jones (1930) reported that bedbugs that took two meals during the third stadium moulted

at a different time from others, but did not say whether this was earlier or later.

It was found that the mortality increased with the decrease in the size and the frequency of blood meals at any instar. Also the longevity of the insects that did not moult at any instar increased with the increase in the size and frequency of feeding.

On Weight Changes during Development

Figs. 9a to 9f show the relationship between the size and the frequency of blood meals and the difference in body weight of the different instars. The first instar was weighed one day after hatching and the other instars on the day of moulting.

The difference in body weight between any two successive instars increases with the increase in the size of the blood meals and with the decrease in the interval between feeding. Similarly, the ratio of the difference in body weight to the total weight of blood meals usually increases with the increase in the size of the blood meal and its frequency. This increase may be due to an increase in the food conversion efficiency with or without a difference in the weight of the unassimilated blood in the gut. The increase in this ratio with the increase in the size of the blood meals when the nymphs were fed every 16 days is mostly due to an increase in the food conversion efficiency.

On the Preoviposition Period

Fig. 10 shows the effect on the preoviposition period. The effect of frequency of feeding depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. In general, the more the insects feed and the shorter the interval between feeding the shorter the preoviposition period.

On Fecundity

The effect of the size and frequency of blood meals on fecundity is shown in fig. 11. Owing to the small number of insects which reached the adult stage as well as their small range in the size and frequency of blood meals, the experiment was completed using adults taken from the standard culture. The size and frequency of blood meals which the adults were fed had a marked effect on the number of females which laid eggs and the number of eggs laid per female. None of the females laid eggs when the feeding period was 15 seconds, at any frequency, nor when fed for 30 seconds every 8th or 16th day. The number of females which laid eggs and the number of eggs laid per female increase with the decrease in the interval between feeding and increases in size of the blood meals. There is a high positive correlation coefficient (0.99) between the weight of blood meals taken by the female and the number of eggs laid per female.

Fig. 12 shows the relationship between the number of eggs laid per milligram of blood and the size of the blood meal and its frequency. The data of the insects reached the adult stage alone did not show clearly the relationship and the correlation coefficient was small (0.45). When analysing these data together with those of the insects taken from the stan-

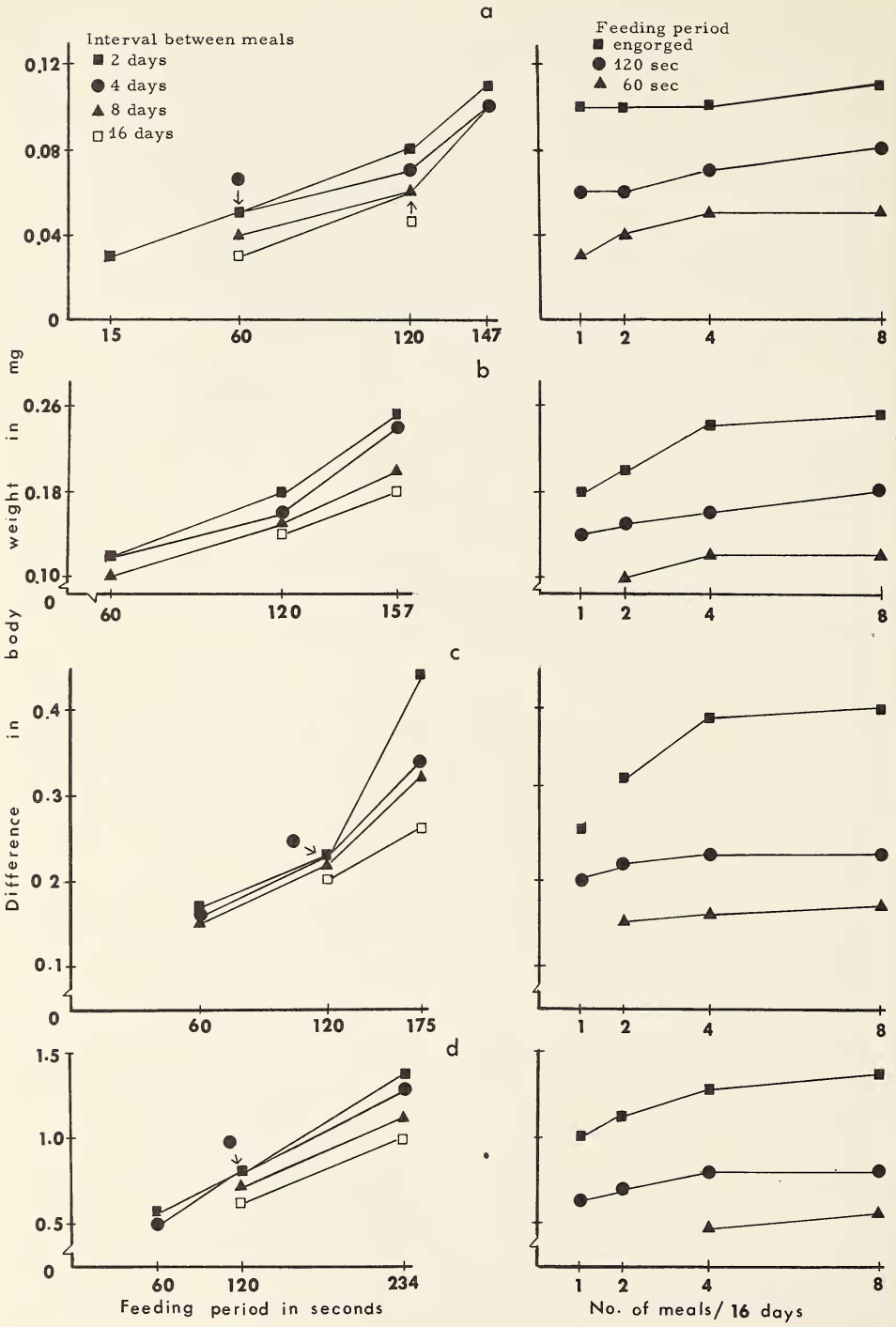


Fig. 9. Effect of the size and frequency of blood meals on the difference in body weight between successive instars of *C. lectularius*. a. 1st to 2nd instar; b. 2nd to 3rd instar; c. 3rd to 4th instar; d. 4th to 5th instar.

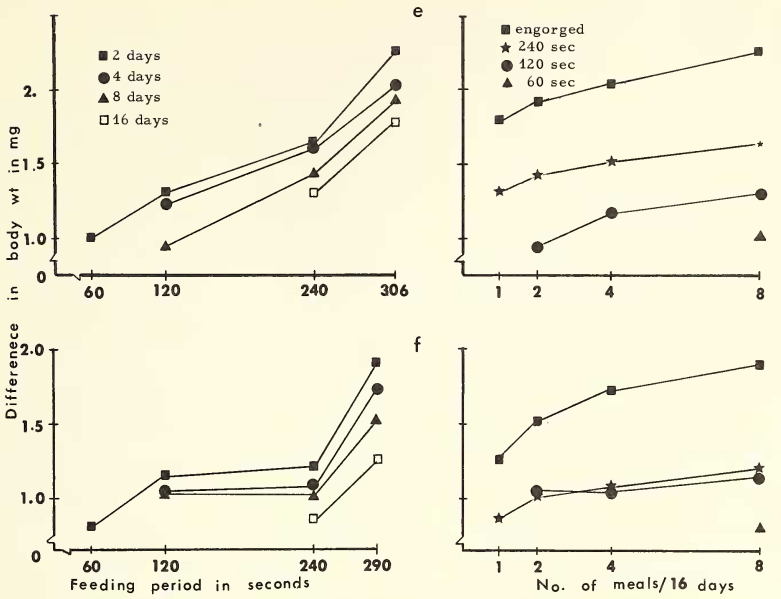


Fig. 9. e. 5th instar and adult female; f. 5th instar and adult male.

dard culture, to complete the experiment, the correlation coefficient was 0.70. The effect of frequency of feeding on the number of eggs laid per milligram of blood (fig. 12a) depends upon the size of the blood meal. The smaller the blood meal the larger the effect of frequency of feeding. When the insects were fed till engorgement the maximum number of eggs/mg of blood was when the insects fed every 4 days. Increasing the frequency of feeding causes a decrease in the number of eggs/mg of blood; the amount of blood taken is then more than enough for optimum egg production. Decreasing the frequency of feeding also decreases the number of eggs/mg of blood, because some of the blood is needed for maintenance metabolism. When the feeding periods were 240, 120, 60, and 30 seconds the number of eggs/mg of blood increased with the increase in frequency of feeding.

Also the effect of the size of the blood meals depends upon the frequency of feeding (fig. 12b). When the interval between meals was 2 days the number of eggs/mg of blood increases with the increase in the size of the blood meal and the optimum egg production was when the feeding period is in the range of 120 to 240 seconds. When the interval between feeding was four days the shape of the curve is changed from convex to step-like with a maximum number of eggs/mg of blood when the insects were fed till engorgement. This indicates that optimum egg production takes place when the feeding period is in the range between 240 seconds and engorgement. The change in the shape of the curve to concave and sigmoid when the intervals between meals were 8 and 16 days respectively shows that taking these blood meals at these intervals was not optimum for egg production and the number of eggs/mg of blood increases with the increase in the size of the blood meal.

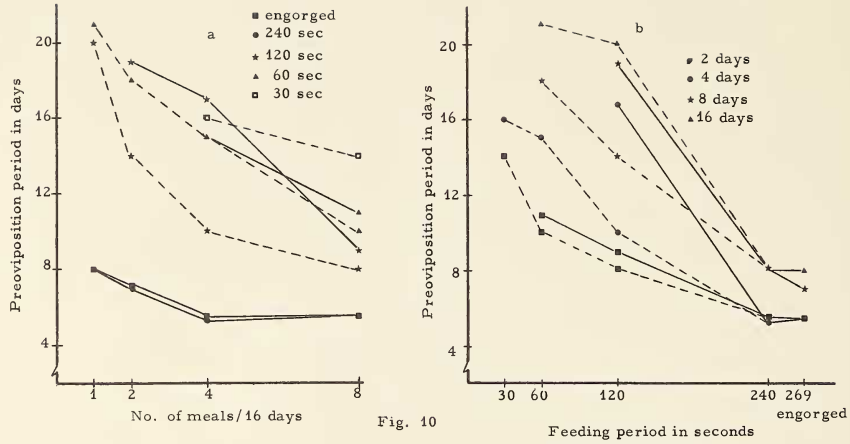


Fig. 10

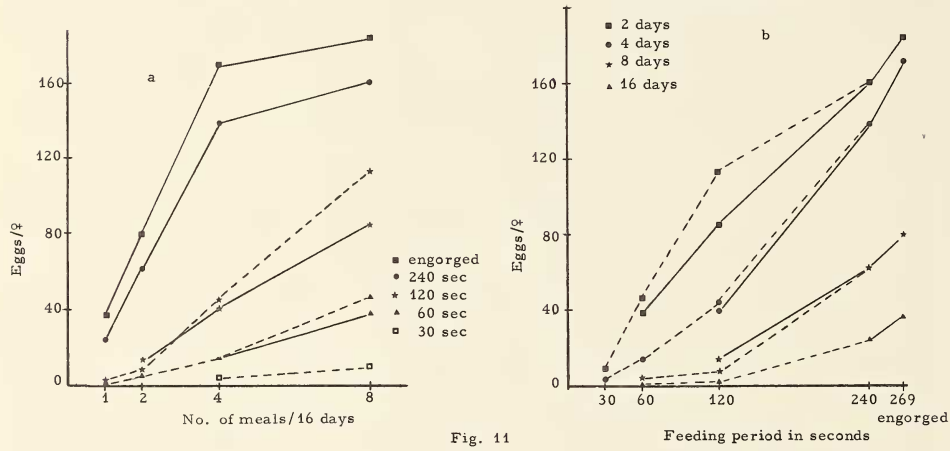


Fig. 11

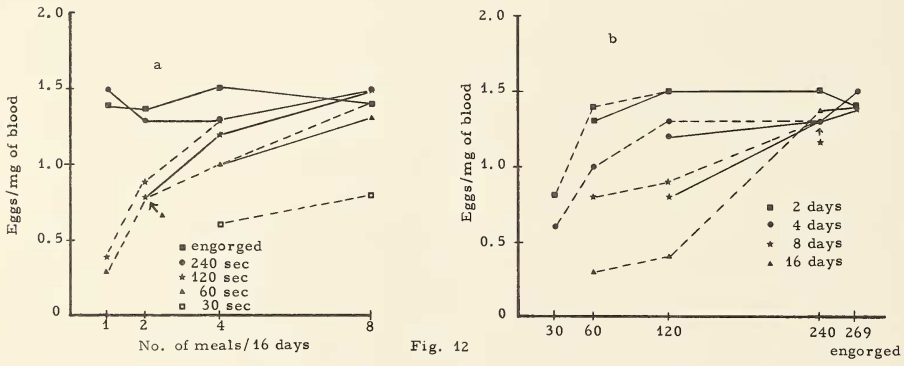


Fig. 12

Figs. 10, 11 & 12. Effects of the frequency (a) and the size (b) of (10) blood meals on the preoviposition period in *C. lectularius*; (11) blood meals on the number of eggs laid per female *C. lectularius*; (12) blood meals on the number of eggs laid per female *C. lectularius*. (--- insects taken from standard culture).

Comparing fig. 5, the relationship between the size of a single blood meal and the number of eggs laid per mg of blood, with the curve connecting size of the blood meal and the number of eggs laid per mg of blood when the interval between feeding was two days (fig. 12b), it is clear that the values when the insects were fed for 60, 120, and 240 seconds are lower than that in fig. 12b. This indicates that the amount of blood responsible for these differences in the values is not directed to egg production but might, for example, stimulate ovarian activity. On the other hand, the number of eggs laid per mg of blood when the insects were fed till engorgement is larger in fig. 5 than in fig. 12b. This indicates that the first feeding period required for optimum egg production is in the range between 240 seconds and engorgement.

There is little correlation (r = 0.32) between the weight of the female after the fifth moult and the number of eggs laid per female. On the contrary, there is a high positive correlation coefficient of 0.97 between longevity and the number of eggs laid per female.

Fig. 13 shows the effect of the mating male's blood meals and their frequencies on the percentage of infertile eggs laid by the females. The smaller the size of the blood meals the larger the effect of frequency of feeding. The correlation coefficient r between the size of the males' blood meals and the percentage of infertile eggs was -0.42 and -0.56 for the data of the insects which reached the adult stage alone, and these data together with those of the insects taken from the standard culture respectively.

The results of the experiments on blood intake and egg production in *C. lectularius* are similar to those reported by Roy (1936) and Friend et al. (1965). Roy (1936) studied *A. aegypti* and found that 0.82 mg of blood was required before the insect would lay eggs. In addition, the number of eggs laid per female was not dependent on the weight of the insect before feeding but there was a good correlation between the size of the blood meal and the number of eggs laid. Friend et al. (1965) found that female *R. prolixus* would not produce eggs until 56.6 mg of blood were fed and the correlation coefficient for the body weight before feeding and egg production was only 0.30.

In some other blood-sucking insects fecundity is influenced mainly by the weight of the female before feeding as long as a certain minimum meal is consumed. Barlow (1955) proposed that the reason for the correlation between the body weight of *Aedes hexodontus* and fecundity is because larger females tended to consume more blood than smaller ones.

On Longevity

Fig. 14 shows the effect of the size and frequency of blood meals on longevity of the female. Statistical analysis of these data showed that the correlation coefficient between the size of blood meals and longevity of the females was 0.97. The longevity of the males increases with the increase in the size of the blood meal and with the decrease in the interval between feeding (fig. 15). Analysis of the data showed a correlation coefficient (r) between the size of the blood meal and longevity of the males of 0.92.

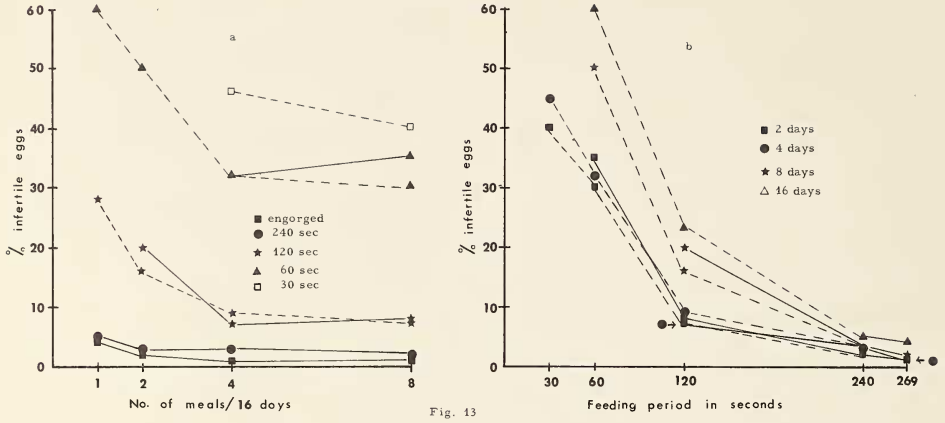


Fig. 13

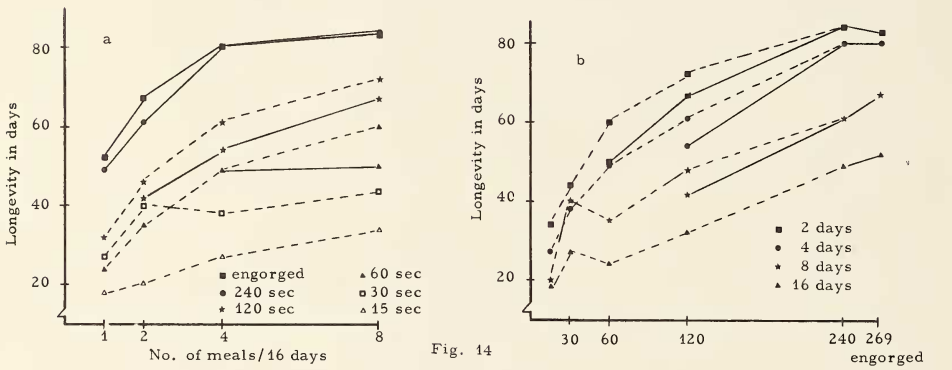


Fig. 14

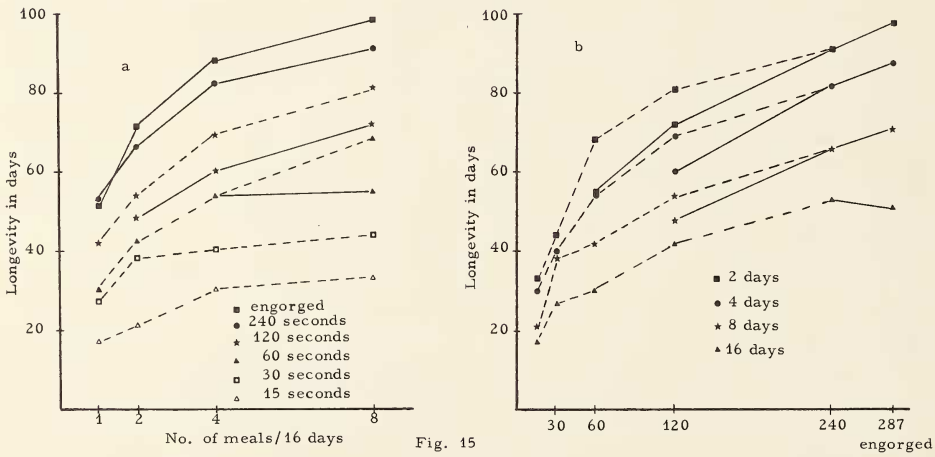


Fig. 15

Figs. 13, 14, & 15. Effects of the frequency (a) and the size (b) of (13) mating male's blood meals on the percentage of infertile eggs laid by female *C. lectularius* ; (14) blood meals on the longevity of female *C. lectularius*; (15) blood meals on the longevity of the male *C. lectularius*. (--- insects taken from standard culture).

PROTEIN CONTENT AND RESPIRATORY RATE IN THE DIFFERENT INSTARS OF *C. LECTULARIUS*

Methods

The protein content in the different instars of *C. lectularius* was determined using Folin phenol reagent (Lowry et al. 1951). Insects used in this experiment were taken from the standard culture. First instar nymphs were taken immediately after hatching and starved for five days. The other nymphal instars were taken on the day of moulting and starved for about ten days. For the adult stage two tests were undertaken. In the first test, fifth instar nymphs were taken from the standard culture and were given a full blood meal and put separately on 2 cm^2 piece of folded filter paper in 2 x 7 cm specimen tubes. After 12 days protein content of the females and males was estimated. In the second test, females and males were put together and given a full blood meal on the second day of moulting. Twelve days later the protein content of the females and males was estimated. The protein content of the eggs was also determined.

Oxygen consumption and carbon dioxide output in the different instars were measured in a Warburg constant volume respirometer using the direct method of Umbreit et al. (1964). Insects used were taken from the standard culture one day after moulting and were given a blood meal before starting the experiments. The respiratory quotient in the different instars was determined.

Results

Protein Content

The protein content in the different instars of *C. lectularius* is shown in fig. 16. The weight of protein per female was greater than that per male. Females which were kept separately had a higher protein content than those which were kept with males. Oviposition seems to be responsible for this difference because the females which were kept with males laid eggs while those which were kept alone did not. Similarly, the difference in protein content of the males may be due to sperm transfer as well as to the activity in courtship and mating. The percentage of protein ranged from 21.8 to 27% in the nymphal instars. In the females it was 26.1 in those which were kept alone and 18.2% in those which were kept with males. In the males it was 27.2 in those kept alone and 25.4% in those kept with females.

Spector (1956) gave the following chemical composition of the human blood:

water	83.000 g/100 ml
total protein	21.800 g/100 ml
lipids	0.560 g/100 ml
carbohydrates	0.439 g/100 ml

From this the amount of protein in the blood meal, in the different instars, was calculated. Lipids and carbohydrates contribute very little in the chemical composition of human blood as compared to protein.

Assuming that there is no conversion from the lipids and carbohydrates of the blood into protein, the efficiency by which the bedbugs, in the different instars, can convert the blood protein into body protein can be estimated (table 4). Protein conversion efficiency in the different instars of *C. lectularius* ranged between 28.0 and 65.3%. Similar results to those of the food conversion efficiency (table 3) would be expected only if the chemical composition of bedbug tissue were similar to that of human blood.

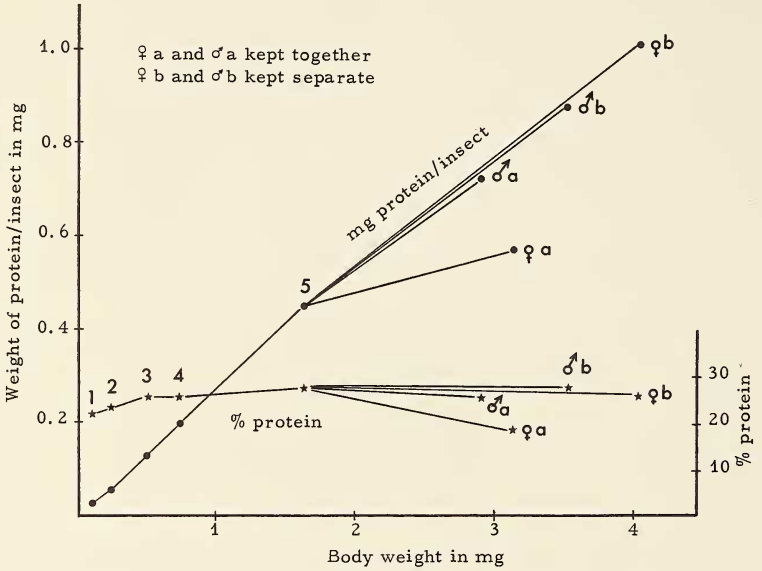


Fig. 16. Relationship between the body weight and protein content in the different instars of *C. lectularius*.

TABLE 4. Protein conversion efficiency in the different instars of *C. lectularius.*

Instar	Wt. of blood meal (mg)	Protein content of blood meal (mg)	Increase in body protein content (mg)	Protein conversion efficiency %
1st	0.26	0.054		
2nd	0.62	0.128	0.035	65.3
3rd	1.09	0.225	0.070	54.8
4th	2.86	0.589	0.068	30.3
5th♀	5.64	1.162	0.256	43.4
5th♂	5.42	1.117	0.603	51.9
			0.313	28.0

Rate of Respiration

The respiratory rates in the different instars of *C. lectularius* are shown in fig. 17. The oxygen uptake per insect per hour increases from the first nymphal instar to the adult stage. Oxygen uptake by the females was greater than by the males. The oxygen uptake, expressed as microliters/milligram of body weight/hour, was the same in the first two nymphal instars and then increased to a maximum in the adult stage. The respiratory quotient in the different instars ranges between 0.88 and 0.95 which indicates that either protein or fat or both are included in the metabolism.

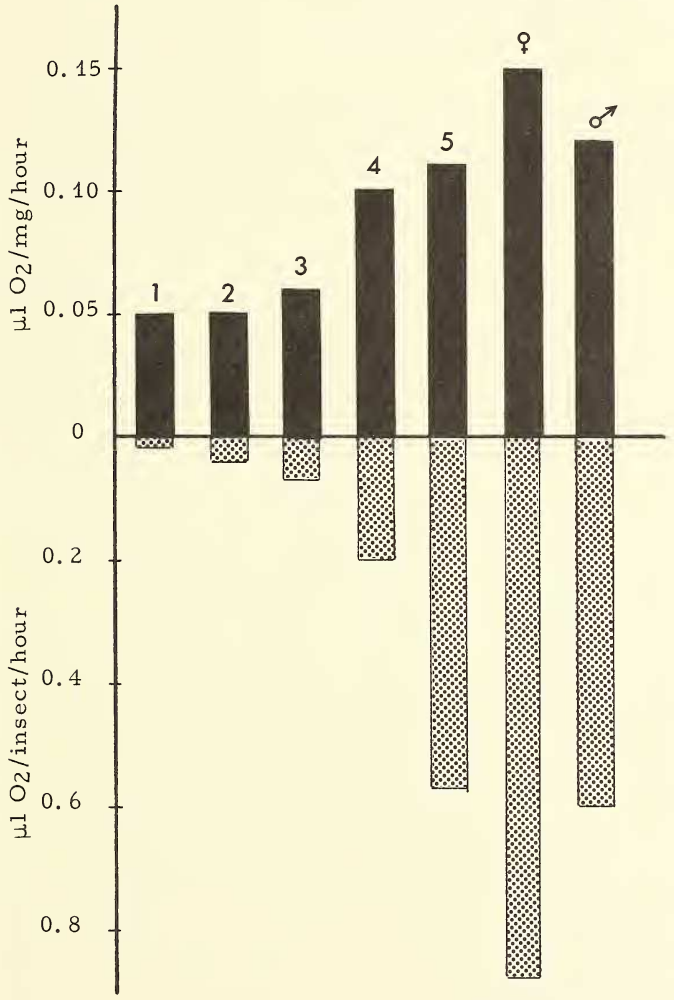


Fig. 17. Oxygen uptake in the different instars of *C. lectularius*.

From the weight of the total protein in the blood meal and the increase of protein content in the different instars, the weight of protein in the blood meal which is metabolized plus that which is not absorbed can be determined. Assuming that there is no interconversion between the chemical constituents of the blood meal during metabolism the weight of protein which is metabolized plus the protein which is not absorbed per day was estimated (table 5). It was found that the rate of metabolism, expressed as mg of protein/day, like that expressed as microliter oxygen/insect/day, increases from the first to the fifth nymphal instar.

TABLE 5. Rate of metabolism in the different nymphal instars of *C. lectularius.*

Instar	Wt. of protein metabolized + protein not absorbed (mg)	Mean longevity (days)	Wt. of protein metabolized + protein not absorbed per day (mg)	Rate of respiration μ1 O_2/insect/day
1st	0.019	34.9	5.4×10^{-4}	0.48 - 0.96
2nd	0.058	63.6	9.1×10^{-4}	0.96 - 1.68
3rd	0.157	63.9	2.5×10^{-3}	1.68 - 4.80
4th	0.333	68.9	4.9×10^{-3}	4.80 - 13.68
5th ♀	0.559	89.1	6.3×10^{-3}	13.68 - 21.12
5th ♂	0.804	89.1	9.0×10^{-3}	13.68 - 14.40

The method by which the weight of protein, which is metabolized plus that which is not absorbed, is shown in table 6. It is clear that the weight of protein which is metabolized plus that which is not absorbed, like the rate of respiration, is greater in the adult female than in any nymphal instar.

TABLE 6. A partial protein budget in the adult female *C. lectularius.*

Weight of blood meal	6.48 mg
Protein content of the blood meal	1.40 mg
Weight of protein/egg	0.034 mg
Number of eggs laid/female	11.55 eggs
Weight of protein in all the eggs	0.393 mg
Weight of protein metabolized + protein not absorbed	1.007 mg
Mean longevity	39.6 days
Weight of protein metabolized + protein not absorbed	2.5×10^{-2} mg/day
Respiratory rate	0.88 μ1 O_2/female/hour

GENERAL DISCUSSION AND SUMMARY

The size of the blood meal has its effect in two ways. The first one is the volume of the blood ingested that causes either stretching of the abdomen and moulting in the nymphal instars or stimulates oviposition in adult females. The second is the quantity of nutrients in the blood ingested and its effects on development or reproduction. It seems that taking a threshold quantity of blood as a single meal is more important to *C. lectularius* than taking the same quantity over a period of time in more than one blood meal. Also the blood of different hosts may have its effect on *C. lectularius* through the differences in the nutritive value of blood.

The results of the effects of the size and frequency of blood meals on *C. lectularius* can be summarized as follows:

1. Moulting can be induced in all the nymphal instars by blood meals smaller than the full blood meal and there is always positive correlation between the size of the blood meal and the percentage of insects moulting.
2. The duration of the nymphal stadia decreases with the increase in the size of the blood meals. It also decreases with the decreases in the number of successive meals required to induce moulting. Neither the size and interval between blood meals nor the number of meals have a significant effect on the period between the last meal and the day of moulting.
3. The food conversion efficiency in the different instars varies between 25.6 and 37.0% and the third instar is less efficient than the others. It takes about 3 to 4 mg of human blood to cause a 1 mg increase in the body weight about 10 days after feeding. Protein conversion efficiency in the different instars ranged between 28.0 and 65.3%.
4. The increase in the size of the females' blood meals causes a decrease in the preoviposition period. The size of the male's blood meal has no significant effect on the preoviposition period of a female mated with him.
5. No eggs were produced by unfed females. The more the females were fed the larger the number of eggs laid per female. There was no significant correlation between the body weight of the female and the number of eggs laid. There was no effect of the size of the mating male's blood meal on the percentage of females laying eggs. The percentage of sterile eggs increased with the decrease in the size of the mating males' blood meals.
6. There is always a significant correlation between the size of the blood meals and the longevity of any instar.
7. It seems important to study the role of crowding on frequency of mating and also the traumatic effects of repeated mating.

ACKNOWLEDGEMENTS

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